FLUORESCENT DIFFERENTIATION OF THE INTERNAL ORGANS AND TISSUES OF INSECTS[1]

ROY J. PENCE

Department of Entomology, University of California, Los Angeles

While studying the effects of shortwave ultraviolet radiation on living termites, it was learned that a strong fluorescence of these insects became apparent whenever they were exposed to certain wave-lengths in the near-ultraviolet portion of the spectrum. In an effort to better observe this interesting phenomenon, three species of termites, along with various other insects and other arthropods, were included.

The source of ultraviolet light found best suited for this study was a commercial mercury vapor lamp of high intensity and pressure emitting most of its light energy in the region of 3650 Angstrom units. This lamp concentrated its illumination into a parabolic beam and was well-suited for low-power microscopy as well as for examination with unaided eye. The shorter wave-lengths in the region of 2537A were also tried but fluorescence in this range was slight.

Of the three species of termites used for experimentation, the subterranean termite, *Reticulitermes hesperus*, responded with the highest degree of fluorescence. Excitation to a slightly lesser degree was found in the drywood termite, *Kalotermes minor*, while the dampwood termite, *Zootermopsis angusticollis*, fluoresced the least of the three. All, however, fluoresced brightly whenever they were placed under the lamp.

Microscopic examination of the termites clearly indicated that only the soft portions of the body fluoresced. The heavier chitin of the head, particularly the head and mandibles of soldiers, was negative. Also the heavier and darker armor of the alates and reproductives gave negative response, with the exception of the connective membranes. Here a strong fluorescence was noticed and became more apparent when the conjunctiva were stretched to expose more of the soft, transparent, chitinous area of these membranes. The dark brown alates of the subterranean termites, with wings attached, reacted negatively when a beam of ultra-violet light was directed down upon them. Those, however, that had broken off their wings showed narrow lines of fluorescence

[1] Submitted for publication on December 11, 1955.

in the areas of connective membranes and, when turned over, emitted a slight fluorescence in the areas where the softer ventral chitin was exposed. It was here that the conjunctiva became strongly excited with a bright blue-white glow and indicated that fluorescence came from beneath the chitin.

In order to determine if the internal content of termites fluoresced, a series of dissections was made. A small embroylogical dish was converted into a dissecting tray. The bottom was filled with melted beeswax to form a soft floor; beeswax was used because of its non-fluorescence factor. A termite was selected, anesthetized, and placed ventral side up. By melting the wax beneath the specimen with a hot point, the body became firmly embedded and ready for dissection. An incision was made just through the chitin wall starting at the vent and progressing anteriorly to the head. The flaps were carefully opened and melted into place in the wax to either side, leaving the body content completely exposed. At the first incision, the adipose tissue emitted a strong bluish-white color, which became noticeable as more of it was laid open. Small bits of chitin were cut away and examined for fluorescence and in all cases found to be negative. It was the adipose tissue, not the chitin, that fluoresced. The following is a brief description of the fluorescence of the body contents of the three species of termites dissected under ultraviolet light using a power of nine diameters of the wide-field miscroscope.

Internal Anatomy

Zootermopsis angusticollis, large worker. The surrounding adipose bodies fluoresced a bright blue-white. In sharp contrast the content of the Malpighian tubules fluoresced a strong yellow; and the tubules could be traced to their origin within the haemolymph and back to their entry into the postventricular region and point of attachment at the pyloric valve. The "ring" of the pyloric valve emitted a fluorenscence of bright blue which became invisible under white light at the same magnification.

Z. angusticollis, reproductive female. Similar to worker with exception of the reproductive system. Here the mature eggs fluoresced a pale yellow and could be followed down to an early developmental stage, when fluorescence became progressively less apparent.

Reticulitermes hesperus, reproductive male. Surrounding adipose tissue fluoresced a brilliant shade of light blue-white, while the thoracic muscles gave off a pale yellow. The gonadal glands stood out above the surrounding adipose tissue and adjacent organs and fluoresced a striking shade of intense blue.

Kalotermes minor, pre-alate. The adipose tissue fluoresced a bright blue-white. Here the digestive tract became evident and clearly established its position and structure by fluorescing a dull orange. The digestive system of the other species examined did not offer the degree of fluorescence as noted in *K. minor.* In an attempt to observe the internal organs located close to the exoskelton of a living, ambulant drywood termite, a group of workers were compelled to ingest nothing but black photographic paper. Others fed upon white paper only. When the termites became engorged with black paper, their digestive systems became black and this was clearly detected by the unaided eye. This enabled the organs such as the Malpighian tubules and portions of the tracheal system to stand out in sharp contrast against the blackened background of the digestive system. In the case of the workers that had eaten white paper, only the adipose tissues fluoresced and they provided no background contrasts.

Although experiments were made with chromatograms in an effort to learn something of the fluorescent content of the body, this approach was set aside due to the complexity of attempting to analyze the Rf of the unknowns when making smears of an entire animal. It can be stated, however, that interesting differences in chromatographical separation and content were noted when smears of the three species of termites were subjected to chromatographic analysis.

In order to learn something about the fluorescence of the internal organs of other insects when dissected, a few were selected for trial. The larval form of a dermestid, *Anthrenus verbasci,* showed a strong fluorescence of bluish-white throughout the adipose tissues. Only a slight trace of contrasting colors could be detected from the exposed internal organs.

An American cockroach, *Periplaneta americana,* created a beautiful display of contrasting colors from the various internal organs. The tracheal system showed up a darker blue than the surrounding adipose tissues. The content of the Malpighian tubules gave forth a strong yellow color that enabled one to trace each tubule to its origin. Again the "ring" of the pyloric valve identified

itself by a bright blue. The gastric caeca gave off a greenish-yellow hue.

In order to include an aquatic insect in the investigation of internal fluorescence, the naiad of a dragonfly, *Progomphus borealis*, was selected. Here but little adipose tissue could be detected, which emitted the characteristic blue-white color. Portions of the intestinal tract also gave off a pale shade of blue. Very little could be seen of the remaining organs.

In examining the difference in fluorescence between the alcoholic specimens of scorpions and whip scorpions (order Pedipalpida) it was found that considerable differences in over-all excitation of the two was apparent. In the scorpion, *Hadrurus hirsutus*, the entire external body fluoresced a brilliant bluish-yellow. In an effort to determine if it was the chitin that fluoresced, one of the heavy scutes was dissected and examined under white light. It was found that the chitin was coated with a thin pigment which may be scraped off with the edge of a blade. Once this pigment is removed, the underlying chitin is negative. On opening the body in order to examine the internal organs, it was found that all body content was negative. However, much of this might be accounted for by the fixative used in the preserving media from which it was taken. By contrast the internal organs of the whip scorpion emitted a strong fluorescence while the external chitinous wall was negative. Here again, as found in the insects examined, the thin connecting chitinous membranes over the joints showed a characteristic degree of fluorescence resulting from the tissues within. From the amount of fluorescing pigment found over the entire body of the scorpion, one is led to speculate on the possible significance of this phenomenon. Could it be that the eyes of scorpions are sensitive to a narrow spectral band in the ultraviolet, as reflected from the moon, and are able to locate their kind by the strong fluorescence that is emitted?

It is felt that more is to be learned in the study of internal anatomy of insects and arthropods through fresh dissections made under the influence of ultraviolet illumination. As it is often the content of an organ rather than the organ itself that fluoresces, and as much of this content is lost through chemical changes when specimens are fixed in some solution, the opportunities for anatomical separation become greater when specimens are observed fresh under this type of illumination It is here that the

identity of small organs, fluorescing differently than their surrounding tissues, and the study of minute differences between any two or more substances, are to be observed that would be otherwise difficult to determine under conventional white light.

References

ELLINGER, P.
1940. Fluorescence microscopy in biology. Biological Review 15(3): 323–350.

DE MENT, J.
1944. Fluorochemistry. Bull. No. 1. Ultra-Violet Prod. Inc., Los Angeles.
1945. Flurobiology. Bull. No. 6. Ultra-Violet Prod. Inc., Los Angeles.

LAWRENCE, R. F.
1954. Fluorescence in Arthropoda. Jour. Ent. Soc. So. Africa 17(2): 167–170.

METCALF, R. L.
1943. A study of riboflavin metabolism in the American roach by fluorescence microscopy. Arch. Biochem. 2:55–62.
1943. The isolation of a red fluorescent pigment from the lampyridol. Ann. Ent. Soc. Amer. 36:37–40.

METCALF, R. L. AND R. L. PATTON
1942. A study of riboflavin metabolism in the American roach by fluorescence microscopy. J. Cell. Comp. Physiol. 19:373–6.
1944. Fluorescence microscopy applied to entomology and allied tissues. Stain Technology 19:11–27.

ON THE DISTRIBUTION OF BOSTRICHOCLERUS BICORNIS VAN DYKE

The clerid beetle, *Bostrichoclerus bicornis* Van Dyke has been known only from the type specimen collected at Palm Canyon, Angel de la Guardia Island, Gulf of California on May 3, 1921, by J. C. Chamberlin. Therefore it is of interest to report that the second known specimen of the species, constituting the first record of its occurrence in the United States, was found in an unidentified lot of clerid material received from Dr. J. N. Belkin of the University of California at Los Angeles. This specimen, a male, was collected in the Iron Mountains, San Bernardino County, California, by R. Zweifel on April 24, 1950.

This significant collection extends the range of *B. bicornis* approximately 365 miles north of the type locality. It now can be assumed that this insect is distributed over an area much more extensive than formerly believed. Most likely it occurs discontinuously in several of the desert mountain ranges in southeastern California, southwestern Arizona, northwestern Sonora and northern Lower California.—W. F. BARR, *University of Idaho, Moscow.*